Functional Perspective of Feeder Organelle from Three-dimensional Ultrastructural Characteristics in Cryptosporidium parvum

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Functional Perspective of Feeder Organelle from Three-dimensional Ultrastructural Characteristics in *Cryptosporidium parvum*

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Abstract

Cryptosporidium is a parasite causing extensive illness both in livestock and humans. Feeder organelle of *Cryptosporidium* is the multi-membranous structures localized on the parasite-host-cell interface that deprives nutrients from host cells. Although the feeder organelle has been summarized as a highly invaginated membranous structure, the three-dimensional fine structure remains unclear. Osmium-maceration procedure for scanning electron microscopy (OS-SEM) is one of the methods to enable visualization of the intracellular ultrastructure including depth direction information after removing soluble proteins. Recently, we investigated and assessed *C. parvum* possessed on the surface of ileal epithelial cells of mice by using transmission electron microscopy (TEM) and OS-SEM. By TEM observation, feeder organelles were recognized as aggregated structures of concentrically-, vertically- and randomly-lined bars. Correspondingly, OS-SEM observation revealed the reticulated network of stacked flat bursiform, ring-shaped bursiform and reticulated tubular membranes. These findings of the three-dimensional ultrastructural characteristics of feeder organelle, which are more intricate than expected, may potentially reinforce the limited knowledge regarding the nature of this attachment interface and the functional mechanisms around extraction of nutrients.

Introduction

Cryptosporidium is a parasite responsible for highly contagious, and cryptosporidiosis in humans and animals (Ryan, Zahedi, & Paparini, 2016). Transmission of *Cryptosporidium* is most often by the fecal-oral route via contaminated water, food or fomites, or by direct ingestion of infected feces, resulting in the intestinal diarrhea (Yoshida *et al*., 2007). Moreover, *C. parvum* is relatively resistant to chlorine at the levels used in potable water. Therefore, cryptosporidiosis infection occurs as a waterborne outbreak with the potential to affect many people at once (Efstratiou, Ongerth, & Karanis, 2017; Mac Kenzie *et al*., 1994; Yamamoto *et al*., 2000). There is no effective medical treatment for either intestinal or biliary cryptosporidiosis, and in AIDS patients, the infection is rarely spontaneously cleared (Chen *et al*., 1998; Theodos, Griffiths, D'Onfro, Fairfield, & Tzipori, 1998). In recent years, *Cryptosporidium* is a life-threatening opportunistic pathogen for children in developing countries containing Africa and Asia, who urgently need specific anti-cryptosporidial therapies (Elfadaly, Hassanain, Hassanain, Barakat, & Shaapan, 2018; HMG *et al*., 2018; Kotloff *et al*.,

2013; Liu *et al*., 2016; Platts-Mills *et al*., 2015). Thus, the infection mechanism of *Cryptosporidium* should be elucidated for the development of effective precaution or treatment of cryptosporidiosis (Ryan *et al*., 2016; Vanathy, Parija, Mandal, Hamide, & Krishnamurthy, 2017).

The infection mechanism of *cryptosporidium* to host cell

Life cycle of *Cryptosporidium* is completed in a single host, and infective sporozoites attach to and invade gastrointestinal epithelial cells to form a unique parasitophorous vacuole on top of the cells (O'Hara & Chen, 2011). The main site of contact between the maturing parasite and the host cell is an extensively folded membrane structure, called the feeder organelle (Zapata, Perkins, Riojas, Wu, & Le Blancq, 2002). The feeder organelle is peculiar and still largely uncharacterized structures (Sharling *et al*., 2010). The feeder organelle is considered as the site at which nutrient uptake from the host cell cytoplasm occurs (Clode, Koh, & Thompson, 2015; Marcial & Madara, 1986; O'Donoghue, 1995). A *Cryptosporidium*-specific ATP-binding cassette, CpABC1, involved in transportation of various molecules (e.g.: metabolites and lipids) across membranes is localized to feeder organelles, indicating that these organelles are important for selective nutrient absorption (Perkins, Riojas, Wu, & Le Blancq, 1999; Zapata *et al*., 2002). The ultrastructure of feeder organelles in *Cryptosporidium* has been examined using transmission electron microscopy (TEM) in murine models of infection and in livestock, and it has been described as "highly invaginated" (Al-Mathal & Alsalem, 2013; O'Hara & Chen, 2011; Pohlenz, Bemrick, Moon, & Cheville, 1978; Rosales, Arnedo, & Mascaró, 1998; Umemiya, Fukuda, Fujisaki, & Matsui, 2005; Valigurová, Hofmannová, Koudela, & Vávra, 2007). The feeder organelle structure was also seen in replicas as multiple membrane facets (Marcial & Madara, 1986). However, how they intake and transport molecules at an organelle level from the host cells remain uncertain.

Osmium-maceration for scanning electron microscopy to visualize 3D ultrastructures of feeder organelles

In contrast to TEM intracellular analysis, scanning electron microscopy (SEM) had been only used to analyze the three-dimensional (3D) surface structure of *Cryptosporidium* (Chen *et al*., 1998; Pohlenz *et al*., 1978; Umemiya *et al*., 2005). The osmium-maceration procedure for SEM (OS-SEM) developed by Tanaka *et al.* in 1981 enables us to make a direct observation the intracellular endomembranous organelles by removing the cytoplasmic soluble proteins selectively from the cracked surface of the cells with diluted OsO_4 solution (Hanaki, Tanaka, & Kashima, 1985; Stowe, Fukudome, & Tanaka, 1986; Tanaka & Mitsushima, 1984; Tanaka & Naguro, 1981). OS-SEM is a very useful method that enables comparative observation with TEM. Recently, we introduced this excellent method, OS-SEM, to visualize the intracellular membranous organelles, especially feeder organelles in *Cryptosporidium* (Bochimoto, Kondoh, Ishihara, Kabir, & Kato, 2019). *Cryptosporidium* oocyst remained on the surface of terminal ileum even after osmium maceration with 0.1% diluted OsO_4 (**Fig. 1A**). Moreover, we confirmed that the present OS-SEM clearly visualizes the intracellular structures of the parasite organelles including endoplasmic reticulum and some types of granules (**Fig. 1B and C**). By utilizing comparative observation of OS-SEM and TEM, we visualize the 3D ultrastructure of feeder organelle possessing three different components at the host-parasite interface: reticulated networks of stacked "dome-shaped" bursiform membranes (concentrically-spread type; cFO): networks of "ring-shaped" bursiform membranes concentrically surrounding "dome-shaped" membranes (vertically-lined type; vFO): reticulated "tube-shaped" membranes (randomly-scattered type; rFO, referred **Fig. 1C**) (Bochimoto *et al*., 2019).

Discussion and future perspectives

Feeder organelles of *Cryptosporidium* have been considered as an invaginated membrane structure that functions to secure a large surface area (Fayer, 2008). However our visualization of the 3D ultrastructural characteristics of feeder organelle indicated that feeder organelles are more intricate and organized than was previously thought (Bochimoto *et al*., 2019). Particularly, randomly tubule-reticular formation of rFO has an similarity to membrane traffic-associated organelles including trans-Golgi network and transitional ER (Hammond & Glick, 2000; Liendo & Joiner, 2000; Pelletier *et al*., 2002). This finding makes us presume that the rFO has specific functions of membrane trafficking of absorbed nutrients (**Fig.1 D**). In the future, the localization of the *Cryptosporidium*-specific transporter, like CpABC1, on each type of the feeder organelle components should be investigated to more clarify the mechanism of transport the nutrients. One of our concerns is that successful parasitism by *Cryptosporidium* needs intricate interactions between the host and the parasite (O'Hara & Chen, 2011). OS-SEM enables to visualize the intracellular 3D ultrastructure in not only *Cryptosporidium* but also the host cells (**Fig. 1C**), which may deal with this concern by comparative observation with TEM. Another concern is that the ultrastructural variation of feeder organelle components might depend on the life cycle stages of *Cryptosporidium*. Monoclonal antibodies, which recognize novel epitopes that could be recently used to define intracellular development (Wilke *et al*., 2018), may resolve this concern. Regarding the structural nature of individual stages, cell attachment and invasion processes, the future lies in 3D imaging as indicated by Clode *et al.* (2015). Ultrahigh-resolution 3D imaging methods could be adopted to ATUMTome sectioning and serial imaging by SEM, block-face serial imaging by SEM, focused ion beam SEM (FIBSEM) and electron tomography by TEM. In addition of these methods, the "long ignored" OS-SEM method is a very powerful tool of analysis of intracellular ultrastructure associated with infection of *Cryptosporidium*.

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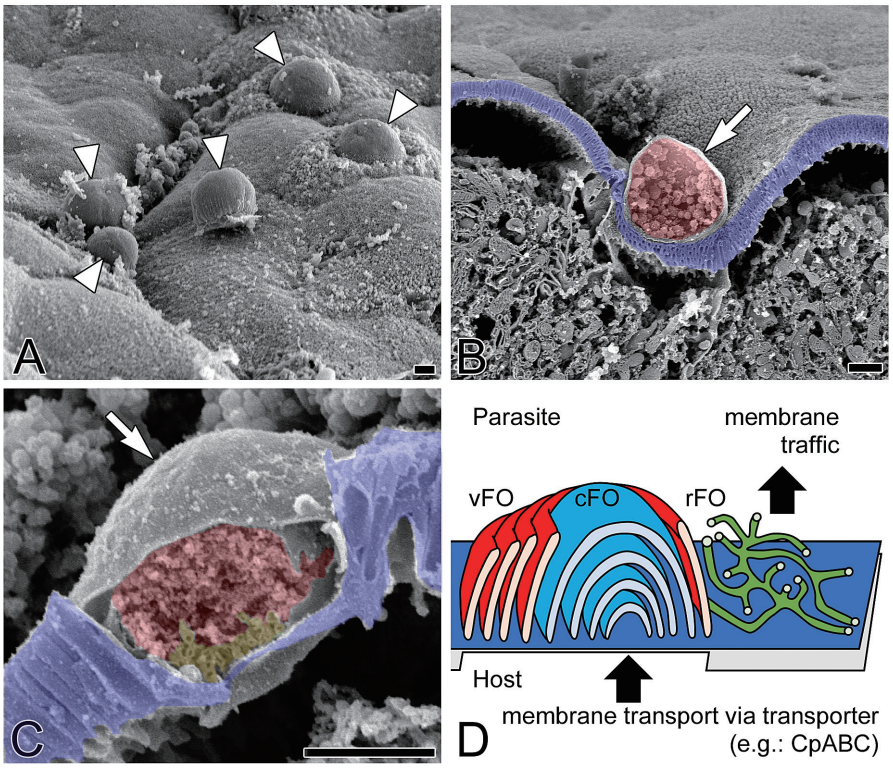

Fig. 1. Osmium-maceration scanning electron microscopic (OS-SEM) images of *Cryptosporidium parvum* attached to mouse ileal cells. [A] Surface of ileal epithelium. Arrowheads indicate outer surfaces images of parasites. [B] Higher magnified image of cross-section of ileal epithelial surface. An arrow indicates the oocyst. Intracellular structures of *Cryptosporidium* are colored red. Microvilli of host cells are colored blue. [C] OS-SEM images of feeder organelle. An arrow indicates the oocyst. Intracellular area and randomly-scattered type feeder organelle (rFO) of *Cryptosporidium* are colored red and green, respectively. Microvilli of host cells are colored blue. [D] Schematic illustration of three-dimensional ultrastructure of feeder organelle. cFO, concentrically-spread type feeder organelle; vFO, vertically-lined type feeder organelle. Bars=1 μm.

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Dedication

We dedicate this paper to the memory of the late Professor Keiichi Tanaka M.D., Ph.D. (1926-2019), who pioneered the field of biological SEM.

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